

MAMMALIAN UROTENSINS II AND APPLICATIONS THEREOF

The present invention relates to mammalian polypeptides, in particular of human or murine origin, having a structure of the urotensin II (UII) type (prepro-urotensin II, pro-urotensin II and urotensin II), and also to applications thereof as a medicinal product, in particular in the form of a composition intended for the treatment of neurodegenerative diseases of traumas to the spinal cord (hemiplegia, paraplegia), and as a tool for screening antihypertensive medicinal products.

The present invention also relates to nucleic acid sequences encoding said polypeptides, to oligonucleotides included in said sequences, and to the use of said sequences as primers and as probes, or for expressing mammalian urotensins II, and in particular human or murine urotensin II.

Urotensin II is a neuropeptide which was first characterized in the urophysis of teleost fish. In these fish, urotensin II is a cyclic peptide comprising 12 amino acids. The characterization of urotensin II in several species of teleost fish has shown that the structure of the C-terminal cyclic heptapeptide is conserved, whereas substitutions are observed in the N-terminal portion of the molecule. This heptapeptide has the following sequence: Cys-Phe-Trp-Lys-Tyr-Cys-Val (SEQ ID NO:9) (1-3).

For many years, it was thought that this peptide was produced exclusively in the urophysis of teleost fish (3), a small neurohemal organ exhibiting similarities with the neurohypophysis, located at the caudal end of the spinal cord; however, it has become apparent that this neuropeptide is not restricted to the caudal neurosecretory system of the fish. It has also been isolated from extracts of trout, skate (4) or lamprey (5) brain. In addition, a peptide similar to fish urotensin II has been detected in the central

nervous system (CNS) of the frog (*Rana ridibunda*) (6) and in a gastropod (*Aplysia californica*) in the cerebral ganglion (7).

5 This peptide, which, in the frog, comprises 13 amino acids, exhibits structural similarities with fish urotensins II, and in particular the cyclic region containing the abovementioned heptapeptide.

10 This neuropeptide also exhibits similarities with somatostatin (2,3); however, fish urotensin II has essentially cardiovascular effects, which can also be observed when this urotensin is administered to mammals, such as rats or rabbits (8,9): contractile effect on arteries (action observed in rats (8) and rabbits (10)), contraction of smooth muscles
15 (spasmogenic effect on certain smooth muscles (bladder and ileum) in amphibians (11)) and effects on cardiac rhythm (observed in amphibians (12)).

20 It has also been shown that fish urotensin II is expressed in the form of precursors, the primary structures of which have been determined using the caudal neurosecretory system of the carp (*Cyprinus carpio*) (13).

25 The inventors have found, unexpectedly, that a urotensin II is expressed in mammals, in particular in humans and in mice, and that, in humans, it can have an activity on motoneuron survival and/or regeneration and on arterial blood pressure (hypertension).

30 A subject of the present invention is polypeptides, isolated from mammals, characterized in that they comprise, at their C-terminal end, a heptapeptide having the following sequence: Cys-Phe, Trp-Lys-Tyr-Cys-Xaa, in which Xaa represents Val or Ile, in that they belong to the urotensin II family and in that they exhibit at least 45%, and preferably at
35 least 70%, similarity with the polypeptide sequence SEQ ID NO:1, corresponding to human prepro-urotensin II.

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The similarity is quantified using the Clustal® program, which is in particular available over the Internet (site <http://www2.ebi.ac.uk/clustalw/>).

The present invention encompasses in particular:

- human prepro-urotensin II (SEQ ID NO:1), human pro-urotensin II (SEQ ID NO:2) and human urotensin II (SEQ ID NO:3),
- rat prepro-urotensin II (SEQ ID NO:30), rat pro-urotensin II (SEQ ID NO:31) and rat urotensin II (SEQ ID NO:32),
- mouse prepro-urotensin II (SEQ ID NO:33), mouse pro-urotensin II (SEQ ID NO:34) and mouse urotensin II (SEQ ID NO:35).

These mammalian polypeptide sequences exhibit, overall, a slight similarity with the fish or frog sequences (Figure 1 and Figure 4):

- 16% similarity between carp prepro-UII- α or prepro-UII- γ and human prepro-II;
- 25% similarity between frog prepro-UII and human prepro-UII.

At the N-terminal of human UII, these sequences exhibit no similarity with the nonmammalian UIIs previously described.

The invention also encompasses polypeptides or peptides derived from mammalian urotensins II and from precursors thereof, according to the invention, by the addition, deletion or substitution of one or more amino acids; they may, for example, be polypeptides into which modifications have been introduced, in particular by substituting dextrorotatory amino acids with levorotatory amino acids (pseudopeptides), or polypeptides which are obtained by molecular modeling and which have urotensin II activity at the neuromuscular junction or other biological targets for urotensin II.

A subject of the present invention is also a purified nucleic acid fragment, characterized in that it comprises all or part of a sequence encoding a

mammalian urotensin II as defined above, or of the sequence complementary thereto, which may be a sense or antisense sequence, with the exception of the EST having the Gen Bank accession number AA535545.

5 In this context, the present invention in particular encompasses the cDNAs, mRNAs and genomic DNAs of the urotensins II and of precursors thereof.

It encompasses the following sequences:

* human sequences:

10 - the sequence encoding human prepro-urotensin II, of sequence SEQ ID NO:4, which comprises 551 bp and in which:

. segment 1-32 is a noncoding sequence,

15 II, segment 33-92 corresponding to the sequence encoding the signal peptide, and

. segment 408-551 is noncoding (see Figure 2);

20 - a fragment of the sequence encoding human prepro-urotensin II (sequence SEQ ID NO:5), characterized in that it encodes human pro-urotensin II, the precursor of human urotensin II, and corresponds to segment 93-407 of SEQ ID NO:4;

25 - a fragment of the sequence encoding human prepro-urotensin II (sequence SEQ ID NO:6), characterized in that it encodes human urotensin II and corresponds to segment 372-407 of the sequence SEQ ID NO:4;

30 - a fragment of the sequence encoding human prepro-urotensin II, which encodes a dipeptide (Pro-Tyr), and which is upstream of the tribasic cleavage site, itself located just upstream of the sequence encoding human urotensin II and specific for said human sequence (see Figure 2);

35 - fragments which can be used as primers consisting of 20 to 50 nucleotides of SEQ ID NO:4, and in particular the sequences SEQ ID NO:7-8 and 10-17, and more particularly the following primer pairs:

. the sequences SEQ ID NO:7 and NO:8, corresponding to segments 267-292 and 535-511, respectively, of the sequence SEQ ID NO:4;

5 . the sequences SEQ ID NO:10 and 11, corresponding to positions 198-216 and 381-404, respectively, of the sequence SEQ ID NO:4;

. the sequences SEQ ID NO:12 and 13, corresponding to positions 46-65 and 214-195, respectively of the sequence SEQ ID NO:4;

10 . the sequences SEQ ID NO:14 (positions 9-28 of the sequence SEQ ID NO:4) and SEQ ID NO:13;

. the sequences SEQ ID NO:15 (positions 14-33 of the sequence SEQ ID NO:4) and SEQ ID NO:13;

15 . the sequences SEQ ID NO:12 and SEQ ID NO:16 (positions 150-131 of the sequence SEQ ID NO:4);

. the sequences SEQ ID NO:17 (positions 8-27 of the sequence SEQ ID NO:4) and SEQ ID NO:13;

20 - fragments which can be used as probes: sequence SEQ ID NO:4 and the fragments consisting of 20 to 50 nucleotides of the sequence SEQ ID NO:4. Said probes are preferably used under the following hybridization conditions:

25 . hybridization: 5X SSPE (0.9 M NaCl/0.05 M sodium phosphate buffer, pH 7.7/0.005 M EDTA), 0.1% SDS, 10X Denhardt's (0.2% Ficoll/0.2% polyvinylpyrrolidone/0.2% BSA), 50 µg/ml tRNA, 50 µg/ml denatured salmon sperm DNA. 37°C, overnight.

30 . washes: 5X SSPE/0.1% SDS, 4 times 5 minutes, room temperature, 3X SSPE/0.1% SDS, 2 times 10 minutes, 30°C.

* rat sequences:

- the sequence encoding rat prepro-urotensin II, of sequence SEQ ID NO:18, which comprises 529 bp and in which:

35 . segment 1-36 is a noncoding sequence,

. segment 37-405 encodes rat prepro-urotensin II, segment 37-96 corresponding to the sequence encoding the signal peptide, and

. segment 406-529 is noncoding (see Figure 3);

- a fragment of the sequence encoding rat prepro-urotensin II (sequence SEQ ID NO:19), characterized in that it encodes rat pro-urotensin II, the precursor of rat urotensin II, and corresponds to segment 96-405 of the sequence SEQ ID NO:18;

- a fragment of the sequence encoding rat prepro-urotensin II (sequence SEQ ID NO:20), characterized in that it encodes rat urotensin II and corresponds to segment 364-405 of the sequence SEQ ID NO:18;

- fragments which can be used as primers consisting of 20 to 50 nucleotides of SEQ ID NO:18, and in particular the sequences SEQ ID NO:36-42, and more particularly the following pairs of primers:

. the sequences SEQ ID NO:36 and SEQ ID NO:37, corresponding to positions 295-314 and 504-485, respectively, of the sequence SEQ ID NO:18;

. the sequences SEQ ID NO:38 (positions 280-299 of the sequence SEQ ID NO:18) and SEQ ID NO:37;

. the sequences SEQ ID NO:39 (positions 131-150 of the sequence SEQ ID NO:18) and SEQ ID NO:40 (positions 314-295 of SEQ ID NO:18);

. the sequences SEQ ID NO:41 (positions 322-341 of the sequence SEQ ID NO:18) and SEQ ID NO:37;

. the sequences SEQ ID NO:42 (positions 50-69 of SEQ ID NO:18) and SEQ ID NO:40;

- fragments which can be used as probes: sequence SEQ ID NO:18 and the fragments consisting of 20 to 50 nucleotides of the sequence SEQ ID NO:18, in particular SEQ ID NO:43 (positions 192-221 of the sequence SEQ ID NO:18).

* mouse sequences

- the sequence encoding mouse prepro-urotensin II, of sequence SEQ ID NO:27, which comprises 539 bp and in which:

. segment 1-36 is a noncoding sequence,

. segment 37-405 encodes mouse prepro-urotensin II, segment 37-96 corresponding to the sequence encoding the signal peptide, and

. segment 406-539 is noncoding (see Figure 4);
- a fragment of the sequence encoding mouse
prepro-urotensin II (SEQ ID NO:28), characterized in
that it encodes mouse pro-urotensin II, the precursor
5 of mouse urotensin, and corresponds to segment 97-405
of SEQ ID NO:27;

- a fragment of the sequence encoding mouse
prepro-urotensin II (sequence SEQ ID NO:29),
characterized in that it encodes mouse urotensin II and
10 corresponds to segment 355-405 of the sequence
SEQ ID NO:27;

- fragments which can be used as primers
consisting of 20 to 50 nucleotides of SEQ ID NO:27, and
in particular the sequences SEQ ID NO:21-26, and more
15 particularly the following pairs of primers:

. the sequences SEQ ID NO:21 and SEQ ID NO:22,
corresponding to positions 295-314 and 485-504,
respectively, of the sequence SEQ ID NO:27;

- the sequences SEQ ID NO:23 (positions 280-299
20 of the sequence SEQ ID NO:27), and SEQ ID NO:22;

- the sequences SEQ ID NO:24 (positions 131-150
of the sequence SEQ ID NO:27) and SEQ ID NO:22;

- the sequences SEQ ID NO:25 (positions 295-314
of the sequence SEQ ID NO:27) and SEQ ID NO:22;

25 - the sequences SEQ ID NO:24 and SEQ ID NO:26
(positions 322-341 of the sequence SEQ ID NO:27);

- fragments which can be used as probes:
sequence SEQ ID NO:27 and the fragments consisting of
20 to 50 nucleotides of the sequence SEQ ID NO:27, and
30 in particular the sequence SEQ ID NO:44 (positions 204-
233 of the sequence SEQ ID NO:27).

The hybridization conditions for the murine
probes are similar to those set out above for the human
sequences.

35 Given the data available to the inventors, it
was not obvious that mammals might express a urotensin
II and that the latter might effectively exert effects
other than cardiovascular effects.

Said polypeptides can be produced either by expressing the nucleic acid sequences as defined above in host cells, or by synthesis, and in particular by synthesis according to the Merrifield technique.

5 A first application of the nucleic acid sequences defined above is to detect either the presence or absence of mRNA encoding a mammalian urotensin II, and in particular human urotensin II, in biological samples (biopsies, for example), especially
10 in individuals with a neurodegenerative pathology or a trauma to the spinal cord, or to detect a mutation in the sequence of the gene, or of the mRNA, encoding urotensin (comparison with the nucleic acid sequences according to the invention).

15 A second application of the nucleic acid sequences defined above is the production of vectors capable of expressing the precursors of human urotensin II, in particular in the context of targeted gene therapy.

20 In the context of these applications, the nucleic acid sequences are advantageously selected from the group consisting of the human sequences SEQ ID NO:4 to SEQ ID NO:6, the rat sequences SEQ ID NO:18 to SEQ ID NO:20 and the mouse sequences SEQ ID NO:27 to
25 SEQ ID NO:29.

A subject of the present invention is also a cell transformed with at least one nucleic acid fragment as defined above.

A subject of the present invention is also
30 pharmaceutical compositions, characterized in that they comprise at least one polypeptide as defined above or at least one nucleic acid sequence encoding all or part of said polypeptides, combined with at least one pharmaceutically acceptable vehicle.

35 For the purpose of the present invention, the term "pharmaceutically acceptable vehicle" is intended to mean both conventional vehicles and those used in the context of gene therapy.

Preferably, said compositions are administered intrathecally.

The compositions according to the present invention make it possible, in particular, to treat
5 neurodegenerative diseases of the spinal cord, in particular diseases of the neuromuscular end-plate, and more particularly amyotrophic diseases, such as amyotrophic lateral sclerosis, or traumas to the spinal cord, more particularly paraplegias and hemiplegias.

10 In an advantageous embodiment of the invention, said compositions are characterized in that the polypeptide is chosen from the group consisting of human prepro-urotensin II (SEQ ID NO:1), human pro-urotensin II (SEQ ID NO:2) and human urotensin II
15 (SEQ ID NO:3), rat prepro-urotensin II (SEQ ID NO:30), rat pro-urotensin II (SEQ ID NO:31) and rat urotensin II (SEQ ID NO:32), and mouse prepro-urotensin II (SEQ ID NO:33), mouse pro-urotensin II (SEQ ID NO:34) and mouse urotensin II (SEQ ID NO:35).

20 In another advantageous embodiment of the invention, said compositions are characterized in that the polynucleotides are selected from the group consisting of the human sequences SEQ ID NO:4 to SEQ ID NO:6, the rat sequences SEQ ID NO:18 to
25 SEQ ID NO:20 and the mouse sequences SEQ ID NO:27 to SEQ ID NO:29.

A subject of the present invention is also the use of polypeptides belonging to the urotensin II family, or of nucleic acids encoding said polypeptides,
30 for preparing a medicinal product intended to treat neurodegenerative diseases of the spinal cord or traumas to the spinal cord.

The polypeptides belonging to the urotensin II family, which can be used in accordance with the
35 invention can originate both from invertebrates and vertebrates, in particular mammals, and preferably human mammals.

In an advantageous embodiment of the invention, said use is characterized in that the polypeptide is

chosen from the group consisting of human prepro-
urotensin II (SEQ ID NO:1), human pro-urotensin II
(SEQ ID NO:2) and human urotensin II (SEQ ID NO:3), rat
prepro-urotensin II (SEQ ID NO:30), rat pro-urotensin
5 II (SEQ ID NO:31) and rat urotensin II (SEQ ID NO:32),
and mouse prepro-urotensin II (SEQ ID NO:33), mouse
pro-urotensin II (SEQ ID NO:34) and mouse urotensin II
(SEQ ID NO:35).

In another advantageous embodiment of the
10 invention, said use is characterized in that the
polynucleotides are selected from the group consisting
of the human sequences SEQ ID NO:4 to SEQ ID NO:6, the
rat sequences SEQ ID NO:18 to SEQ ID NO:20 and the
mouse sequences SEQ ID NO:27 to SEQ ID NO:29.

15 A subject of the present invention is also a
diagnostic kit, characterized in that it comprises at
least one sequence as claimed in the invention, capable
of detecting the presence of an mRNA, possibly
modified, encoding a mammalian urotensin II, in a
20 biological sample.

A subject of the present invention is also the
use of said polypeptides, which also have hypertensive
activity, for selecting antagonists of this activity
(selection of antihypertensives having activity against
25 urotensins II as claimed in the invention).

Besides the preceding arrangements, the
invention also comprises other arrangements, which will
emerge from the following description, which refers to
examples of implementation of the process which is the
30 subject of the present invention, and also to the
attached drawings, in which:

- Figure 1 illustrates the alignment of the
deduced amino acid sequences of, respectively, human,
frog and carp prepro-UII. In this figure, the signal
35 sequence is indicated in italics; the conserved amino
acids are indicated in black; the cleavage sites of the
prohormone are indicated by stars and the conserved
amino acid residues are indicated by a black circle.
The disulfide bridge present in the UII sequence is

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indicated under the urotensin II sequence. The amino acids are numbered on the right of the figure;

- Figure 2 illustrates the structure of human prepro-UII, pro-UII and UII;

5 - Figure 3 illustrates the structure of rat prepro-UII, pro-UII and UII;

- Figure 4 illustrates the structure of mouse prepro-UII, pro-UII and UII;

10 - Figure 5 illustrates the tissue distribution of human prepro-UII mRNA. Figure 5A illustrates the dot blot analysis of the expression of prepro-UII mRNA in various human tissues, using the Clontech Masterblot (poly(A) RNA from 50 different human tissues (80-448 ng/point, standardized using the level of RNA
15 expression of 8 housekeeping genes). The positive controls consist of human genomic DNA; the negative controls include DNA or RNA from yeast or from *E. coli*, and also human repeat genomic sequences (H). The blot is hybridized with the probe of cDNA encoding human
20 prepro-UII, and exposed to an X-Omat film for 2 days. Figure 5B illustrates the Northern Blot analysis of the expression of prepro-UII mRNA in the human spinal cord; 2 µg of spinal cord poly(A) mRNA are hybridized with the probe consisting of the human prepro-UII cDNA. The
25 size is determined using RNA size markers (calibrated standard nucleotide chains). Figure 5C corresponds to X-ray autoradiographs and shows the distribution of prepro-UII mRNA in the human spinal cord. The frontal sections are hybridized with an antisense (1) or sense
30 (2) prepro-UII riboprobe, and exposed to X-ray-sensitive films for 10 days;

- Figure 6 is a comparison of the primary structures of urotensin II from various species. Dashes have been inserted in order for the sequences to be
35 optimally aligned. The dots illustrate the amino acids residues which are identical between the various sequences, with respect to the human sequence;

- Figure 7 illustrates the tissue distribution of rat and of mouse prepro-UII mRNA.

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It should be clearly understood, however, that these examples are given merely as an illustration of the subject of the invention, of which they in no way constitute a limitation.

5 **EXAMPLE**

- Materials and methods

* Isolation of the human prepro-UII cDNA:

An EST (expressed sequence tag) sequence encoding a peptide having a certain identity with frog urotensin II is registered under the no. AA535545 (Genbank). This sequence derives from an EST analysis of cDNA clones obtained from colon tumors.

Two primers (5'-AACCCAAGAGGAAATTTGAGAAAGTT-3' (SEQ ID NO:7) and 5'-CCAGGTAACAATGAACAGGGTGTAG-3' (SEQ ID NO:8)) deduced from the EST sequence enable a 269 bp fragment to be synthesized by RT-PCR from a human colon tumor sample, under the following conditions:

94°C, 4 min, 1X; 94°C, 1 min; 55°C, 1 min; 72°C, 1 min, 30X; 72°C, 5 min, 1X.

The PCR product is labeled with [³²P] dCTPs by random priming, and then hybridized with various human tissues containing poly(A) RNAs and also with positive and negative controls (MasterBlot, Clontech, Palo Alto). The hybridization and washes are carried out under the following conditions:

* prehybridization: incubation at 42°C, at least 5 hours in a reaction medium comprising:

50% formamide, 5X SSC, 5X Denhardt's, 50 mM NaH₂PO₄/Na₂HPO₄, 200 µg/ml salmon sperm DNA, 0.1% SDS.

* hybridization: the same medium as the prehybridization medium, with the labeled probe in addition.

* washes: 4 times 5 minutes at room temperature, 2X SSC + 0.1% SDS, and then twice 10 minutes at 42°C, 0.1% SDS + 0.1% SDS.

The blot is exposed against an X-OMAT film (Kodak) and the hybridization signals are quantified using the Densylab program (Bioprobe Systems, France).

The strongest hybridization signal is obtained in the spinal cord.

Under these conditions, poly(A) RNA from human spinal cord (Clontech) is used to amplify the 5' end of the human UII cDNA using a RACE kit (Marathon cDNA amplification kit, Clontech).

* Northern blot analysis (RNA transfer onto membrane):

2 µg of poly(A) RNA from human spinal cord (Clontech) are loaded onto an agarose-formaldehyde gel; after migration and transfer onto nylon membrane, hybridization is carried out with the PCR product specific for the human UII cDNA, labeled by incorporation of [³²P] dCTP.

15 * In situ hybridization:

Sense and antisense human riboprobes are prepared by *in vitro* transcription of the PCR products obtained with specific prepro-UII primers, 5'-CTGCCAGAGATGCTGGGTG-3' (SEQ ID NO:10) and 5'-GACACAGTATTTCCAGAAGCAATC-3' (SEQ ID NO:11), extended at their 5'-terminal end with the SP6 and T7 promoters of the corresponding RNA polymerases; the transcription is carried out in the presence of [³⁵S]UTP (Amersham) or of digoxigenin-11-UTP (Boehringer), and of T3 or T7 RNA polymerase, under the same PCR conditions as those set out above.

A portion of human cervical spinal cord was obtained by autopsy from a 70-year-old male.

The tissue fragment is fixed in 4% formaldehyde for 24 hours, embedded in Tissue-Tek and frozen in liquid nitrogen.

Frontal sections (12 µm thick) are cut using a cryostat and stored at -80°C.

The sections are pretreated as described in H. Tostivint et al. (14) and covered with a prehybridization buffer (50% formamide, 0.6 M NaCl, 10 mM Tris-HCl, pH 7.5, 0.02% Ficoll, 0.02% polyvinylpyrrolidone, 0.1% BSA, 1 mM EDTA, pH 8.0,

550 µg/ml of denatured salmon sperm DNA and 50 µg/ml of yeast tRNA).

The hybridization is carried out at 55°C overnight in the same buffer (with the exception of the concentration of denatured salmon sperm DNA: 60 µg/ml), supplemented with 10 mM of dithiothreitol, 10% dextran sulfate and heat-denatured riboprobes.

The ³⁵S-labeled probes and the digoxigenin-labeled probes are diluted in the hybridization buffer so as to obtain a final concentration of 5x10⁶ dmp/ml and 1:100 (v/v), respectively.

The sections are washed in 2X SSC buffer at 60°C and treated with RNase A (50 µg/ml) for 60 min at 37°C.

Five washes under stringent conditions are carried out in a 0.1X SSC, 14 mM β-mercaptoethanol, 0.05% sodium pyrophosphate buffer at 60°C.

The sections hybridized with the ³⁵S-labeled riboprobes are dehydrated in solutions of ethanol comprising increasing concentrations of 0.3 M sodium acetate, and exposed on a Hyperfilm-βmax film (Amersham) for 2 weeks.

The sections hybridized with the digoxigenin-labeled riboprobes are washed in a buffer 1 (100 mM Tris-HCl and 150 mM NaCl, pH 7.5), incubated for 30 min in a blocking buffer (2% of Boehringer blocking agent in buffer 1) and incubated for 2 hours in buffer 1 containing 1:500 of alkaline phosphate-conjugated anti-digoxigenin antibodies (Boehringer), 1% of normal sheep serum and 0.1% of Triton X100. The sections are rinsed twice, for 10 min in buffer 1 and 10 min in buffer 2 (100 mM Tris-HCl, 100 mM NaCl and 50 mM MgCl₂, pH 9.5), and then incubated for 3 hours in a chromagenic solution consisting of Fast Red TR/Naphthol AS-MX and 3 mM Levamisole (Sigma).

The reaction is stopped by rinsing in a TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0).

The sections are examined under a microscope (Leitz Orthoplan).

* Sequencing

The amplification product is subcloned into a pGEM-T vector (Promega) and sequenced with the SP6 and T7 primers using the Amersham sequencing kit (Thermo Sequenase).

- Results

* Characterization of the human prepro-UII cDNA:

The open reading frame of the human UII precursor cDNAs encodes a 124 amino acid protein (Figure 1 and Figure 2).

The organization of the human UII precursors is similar to that of the carp UII prohormone and to that of the frog UII precursor. All these precursors comprise an N-terminal signal sequence and then a flanking peptide, a proteolytic cleavage site (Lys/Arg-Lys-Arg) and the urotensin II sequence located at the C-terminal end of each precursor.

The N-terminal flanking peptides of the carp, frog and human precursors exhibit virtually no similarity.

The human UII comprises only 11 amino acids, whereas the frog and carp UII have 13 and 12 amino acids, respectively (Figure 6).

The sequence of the C-terminal cyclic heptapeptide of urotensin II is conserved in the frog and in humans. On the other hand, the N-terminal region of the peptide is very variable.

In the frog, as in the carp, the C-terminal region of the flanking peptide contains a dibasic potential cleavage site (Arg-Lys and Arg-Arg) which might generate the conserved dipeptide Gln-Phe.

However, in humans, the sequence of the corresponding dipeptide is totally different (Pro-Tyr) (Figure 1 and Figure 2).

* Distribution of the human prepro-UII mRNA was studied:

The tissue distribution of the human prepro-UII mRNA was studied by dot blot analysis (Figure 5A).

5 Out of the 50 different tissues tested, the spinal cord shows the strongest hybridization signal. The prepro-UII mRNA is also observed in the *medulla oblongata*, but the strength of the signal is much weaker than that obtained in the spinal cord.

10 In the peripheral tissues, the presence of prepro-UII mRNA is detected in the kidney, spleen, small intestine, thymus, prostate, hypophysis and adrenal gland, and in smaller amounts, in the stomach, pancreas, ovaries and liver (Figure 5A).

15 The analysis by Northern blot reveals the presence of a single band corresponding to a prepro-UII mRNA of approximately 700 bp in the human spinal cord.

The labeling of sections of the cervical portion of the human spinal cord by *in situ* hybridization shows that the prepro-UII mRNA is located
20 in the motoneurons (Figure 5C).

* Characterization of the rat, and of the mouse, prepro-UII cDNA:

The open reading frame of the rat and mouse UII precursor cDNAs encodes a 123 amino acid protein
25 (Figures 3 and 4).

Figure 7 illustrates the results of the distribution in various rat and mouse tissues, using RT-PCR. The total RNAs are extracted and subjected to
30 an RT-PCR reaction, under conditions similar to those set out above.

In Figure 7A, the rat (left) and mouse (right) PCR products are detected by hybridization with an internal oligonucleotide probe specific for rat and for
35 mouse (the sequences SEQ ID NO:43 and 44, respectively).

Figure 7B illustrates GADPH PCR products, used as a control to reflect equivalent RNA levels, loaded onto an agarose gel.

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As emerges from the above, the invention is in no way limited to its methods of implementation, preparation and application which have just been described more explicitly; on the contrary, it encompasses all of the variants thereof which may occur to a person skilled in the art, without departing from the context or scope of the present invention.

CLAIMS

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- 1) A polypeptide, isolated from mammals, characterized in that it comprises, at its C-terminal end, a heptapeptide having the following sequence: Cys-Phe,Trp-Lys-Tyr-Cys-Xaa, in which Xaa represents Val or Ile, in that it belongs to the urotensin II family and in that it exhibits at least 45%, and preferably at least 70%, similarity with the polypeptide sequence SEQ ID NO:1, corresponding to human prepro-urotensin II.
- 2) The mammalian polypeptide as claimed in claim 1, characterized in that it is selected from the group consisting of the human sequences SEQ ID NO:1-3, of the rat sequences SEQ ID NO:30-32 and of the mouse sequences SEQ ID NO:33-35.
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- 3) A purified nucleic acid fragment, characterized in that it comprises all or part of a sequence encoding a polypeptide as claimed in claim 1 or claim 2, or of the sequence complementary thereto, which may be a sense or antisense sequence, with the exception of the EST having the Gen Bank accession number AA535545.
- 4) The nucleic acid fragment as claimed in claim 3, characterized in that it is selected from the group consisting of the sequences SEQ ID NO:4-6, the sequences SEQ ID NO:18-20 and the sequences SEQ ID NO:27-29.
- 5) A recombinant vector, characterized in that it contains a nucleic acid fragment as claimed in claim 3 or claim 4.
- 6) A cell transformed with at least one nucleic acid fragment as claimed in claim 3 or claim 4.
- SUB
A3
- 7) A reagent for detecting a nucleic acid fragment as claimed in claim 3 or claim 4, characterized in that it comprises between 20 and 50 nucleotides of the sequence SEQ ID NO:4, of the sequence SEQ ID NO:18 or of the sequence SEQ ID NO:27.

8) The reagent as claimed in claim 7, characterized in that it is selected from the group consisting of:

- a fragment of the sequence encoding human prepro-urotensin II, which encodes a dipeptide (Pro-Tyr), and which is upstream of the tribasic cleavage site, itself located just upstream of the sequence encoding human urotensin II and specific for said human sequence;

- fragments which can be used as primers: SEQ ID NO:7 and NO:8, SEQ ID NO:10-17; SEQ ID NO:21-26; SEQ ID NO:36-42, and

- fragments which can be used as probes: sequence SEQ ID NO:4 and the fragments consisting of 20 to 50 nucleotides of said sequence SEQ ID NO:4; sequence SEQ ID NO:18 and the fragments consisting of 20 to 50 nucleotides of said sequence SEQ ID NO:18, and sequence SEQ ID NO:27 and the fragments consisting of 20 to 50 nucleotides of said sequence SEQ ID NO:27.

9) A pharmaceutical composition, characterized in that it comprises at least one polypeptide as claimed in either of claims 1 and 2, or one nucleic acid sequence as claimed in either of claims 3 and 4 encoding all or part of said polypeptides, combined with at least one pharmaceutically acceptable vehicle.

10) The use of polypeptides belonging to the urotensin II family, or of nucleic acid sequences encoding said polypeptides, for preparing a medicinal product intended to treat neurodegenerative diseases of the spinal cord or traumas to the spinal cord.

11) A process for detecting the presence or absence of an mRNA encoding a mammalian urotensin II, in particular in individuals with a neurodegenerative pathology or a trauma to the spinal cord, by bringing a suitably treated biological sample into contact with at least one reagent as claimed in claim 7 or claim 8.

12) A process for detecting a mutation in the sequence of the gene or of the mRNA encoding urotensin, characterized in that it comprises extracting said DNA

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A4

SUB
A4

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A5

13) A diagnostic kit, characterized in that it
5 comprises at least one sequence as claimed in either of
claims 3 and 4, capable of detecting the presence of an
mRNA, possibly modified, encoding a mammalian urotensin
II, in a biological sample.

14) The use of the polypeptides as claimed in claim
10 1 or claim 2, for selecting anti-hypertensives.

ADD \rightarrow add c4
A⁶

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80	81	82	83	84	85	86	87	88	89	90	91	92	93	94	95	96	97	98	99	100
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80	81	82	83	84	85	86	87	88	89	90	91	92	93	94	95	96	97	98	99	100